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Development and validation of analytical method for simultaneous determination of curcumin and capsaicin in bulk

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Abstract
Curcumin and Capsaicin are the natural chemical constituents having potent and synergistic anti-inflammatory activity in combination. In the present analytical research work an attempt has been made to develop and validate simple, selective, specific, precise UV-Spectrophotometric method for simultaneous determination of Curcumin and Capsaicin in bulk. UV-Spectrophotometric method was developed by using methanol as solvent in which Curcumin and Capsaicin showed maximum absorbance at 421 nm and 280 nm respectively. The developed analytical method was validated as per International Conference Harmonization guidelines. Analytical method was found to be linear between the concentration range of 1-5 µg/ml and 25µg-125µg/ml respectively for Curcumin and Capsaicin and also method was found to be selective, specific, precise, robust and rugged with % RSD less than 2%. Hence the newly developed analytical method can be used for simultaneous determination of Curcumin and Capsaicin and its routine quality control analysis in combined bulk.

Keywords: Curcumin, capsaicin, anti-inflammatory, methanol, analytical, uv-spectrophotometry

Introduction
The traditional medicines play an important role in human being from the ancient days and they are also known as "Traditional Healers". Many plant and its chemical constituents are reported to have many pharmacological effects. Out of such traditional medicines Curcumin (Figure 1) and Capsaicin (Figure 2) [3] are important chemical constituents having many pharmacological activities. Curcumin is obtained from various species of *Curcuma* such as *Curcuma longa*, *Curcuma amada*, *Curcuma aromatica*, *Curcuma aeruginosa*, *Curcuma malabarica*, *Curcuma caesia* and *Curcuma longa* [1] and also Capsaicin shows anti-inflammatory activity which is major chemical constituent obtained from Capsicum (Hot peppers). [2] Both the species of *Capsicum* and *Curcuma* is considered as one of most promising drug as Anti-inflammatory. Hence there is need to design and develop an effective pharmaceutical formulation containing both Curcumin and Capsaicin for its synergistic anti-inflammatory activity and also there is need to develop and validate a newer analytical technique to analyze both Curcumin and Capsaicin in combined bulk and its dosage forms.

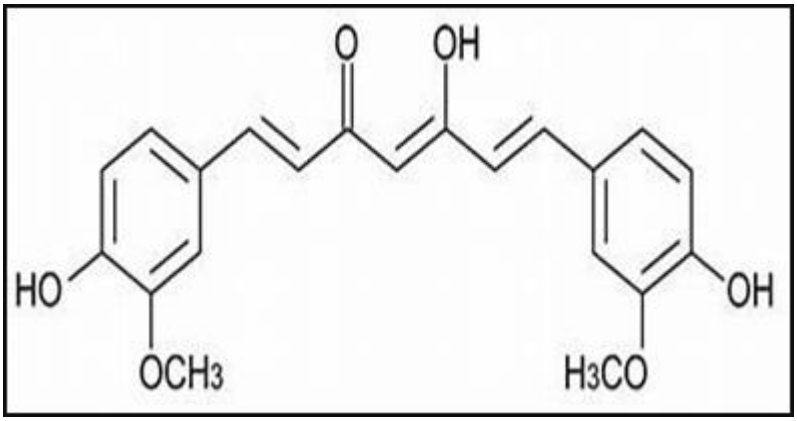

Fig 1: Structure of Curcumin

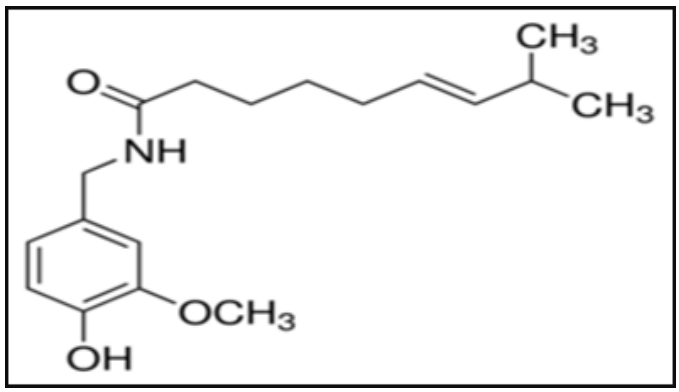

Fig 2: Structure of Capsaicin

Materials and Method

Chemicals and Reagents

The Curcumin and Capsaicin was obtained from Natural Remedies Pvt. Ltd. Bengaluru. Analytical grade methanol of SDFCL and Fisher Scientific was used

Instrumentation

Sican 2301 UV-Spectrophotometer and quartz cells having path length of 10 mm was used for analysis.

Analytical method development

Selection of Solvent

Solubility of Curcumin and Capsaicin was checked in different solvents by performing practically as well as through literature search and it was observed that both drugs showed free solubility in methanol and hence it was used as solvent for development of UV-Spectrophotometric method.

Selection of wavelength of maximum absorbance

Curcumin and Capsaicin was dissolved in methanol and scanned between the 800-200 nm ranges using UV-Visible Spectrophotometer. The Curcumin and Capsaicin was showed maximum absorbance at 421nm and 280nm respectively and hence these wavelengths were selected for analysis.

Preparation of stock solution and serial dilutions

10 mg of Curcumin and Capsaicin was weighed accurately and transferred into two different 10 ml volumetric flasks and dissolved in Methanol separately. The volume made up to the mark to get 1000 µg /ml of Curcumin and Capsaicin. From the above stock solution further serial dilutions having 1-5 µg /ml and 25-125 µg /ml of Curcumin and Capsaicin was prepared respectively.

Method Validation

Newly developed analytical method was validated as per the ICH guidelines by using typical parameters such as specificity, selectivity, linearity and range, precision, robustness, ruggedness, limit of detection (LOD), limit of quantification (LOQ) and solution stability.[4,5,6]

Specificity and Selectivity

UV-Spectrum of methanol, Curcumin and Capsaicin was obtained by scanning the solvent and both drug solutions between 800-200 nm.

Linearity and Range

Solutions containing1-5 µg/ml of Curcumin and 25-125 µg/ml Capsaicin were scanned in triplicates and absorbances are measured and the standard calibration curve was obtained by Absorbance Vs Concentration and correlation coefficient and linear regression were calculated.

Precision

Precision was performed in terms of system, intraday and interday precision. System precision was done by checking the absorbances of six replicates of combined solutions having 3µg/ml of Curcumin and 75µg/ml of Capsaicin and % Relative Standard Deviation (RSD) for absorbances was calculated. Intraday precision was performed by checking the six replicates of combined solutions having 3µg/ml of Curcumin and 75µg/ml of Capsaicin and %RSD for absorbances was calculated at four different times in a day independently. Interday precision was performed by same procedure as mentioned in system precision on two different days independently.

Robustness

The robustness was performed by deliberate small change in the wavelengths of analysis. The absorbances of solutions having Curcumin and Capsaicin at 420 nm and 279 nm, 422nm and 281 nm respectively calculated.

Ruggedness

Ruggedness was carried out by different analyst on different day, by different instruments (Shimadzu1900) and by using different solvent make (Scientific Fisher). Absorbances of six replicate solutions having Curcumin and Capsaicin were analysed by different analyst on different day and in different instrument and also by utilizing methanol of different make as solvent. % RSD for each was calculated.

Limit of Detection and Limit of Quantification:

Statistical method was used to determine Limit of Detection (LOD) and Limit of Quantification (LOQ). The values were obtained from the data was reported.

Solution Stability

The solution stability was carried out by keeping the prepared stock solution of both drugs for longer time (20 days) and analyzing the each solution on different time intervals.

Results and Discussion

Method development

For the method development first solubility of Curcumin and Capsaicin was checked in various solvents. According to Literature reviews and practical analysis it was observed that both drugs are freely soluble in Methanol and also spectrum of those drugs in Methanol showed very good comparing to other Solvents.

Selection of wavelength of detection

The working standard solutions containing Curcumin and Capsaicin showed maximum absorbance at 421 nm and 280 nm respectively and spectrums were showed in Figure 3 and 4 and developed method parameters were presented in Table 1.

Method validation

Specificity and selectivity

It was observed that there is no absorbance of methanol at UV-Spectrum of both analytes and hence method was found to be specific and selective. UV-Spectrum of methanol was showed in Figure 5.

Linearity and Range

The method was shown linearity between the concentration range of 1µg/ml- 5µg/ml and 25µg/ml- 125µg/ml with correlation coefficient of 0.998 and 0.998 respectively. Linearity and range data of both analytes was presented in Table 2.

Precision

The % RSD of absorbances obtained in each replicates were calculated for system precision, intraday and interday precision and it was observed that precision at each level was found to be less than 2% and hence developed method was found to be precise (Table 3, 4, 5).

Robustness

In the robustness parameter, in both Curcumin and Capsaicin wavelengths was altered deliberately to lower and higher λ Max, there was no any difference in absorbance and % RSD was found to be at lower wavelength 1.287% & 1.068% and 0.616% & 1.841% at higher wavelength respectively. Hence the developed method was found to be robust and the results were presented in Table 6.

Ruggedness

The % RSD of absorbances of Curcumin and Capsaicin by different analyst, different instrument and different solvent make was found to be less than 2% Table 7.

LOD and LOQ

The LOD values of Curcumin and Capsaicin were found to be 0.225 µg/ml & 7.380 µg/ml and LOQ values of Curcumin and Capsaicin were found to be 0.68µg/ml & 22.365µg/ml respectively by statistical calculations.

Solution Stability

The solution stability was proved for 360 hrs and results are showed in Table 8.

Method validation report was presented in Table 9.

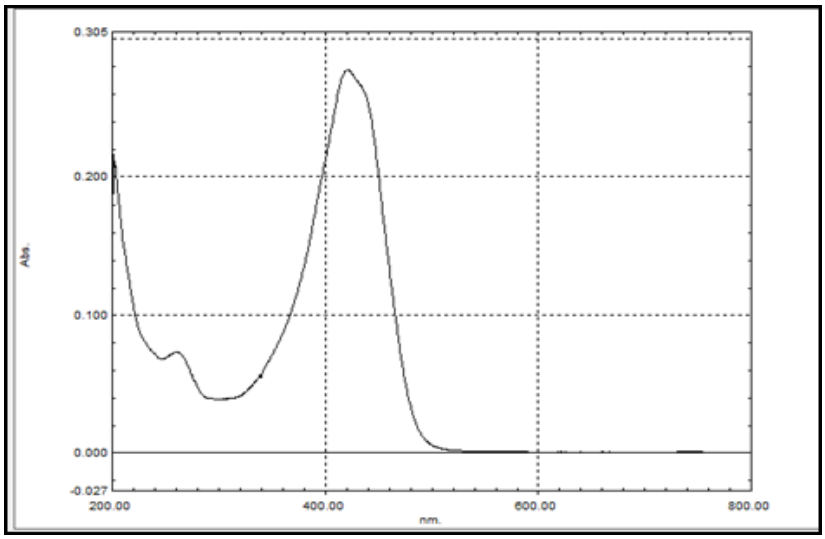

Fig 3: UV-Spectrum Curcumin

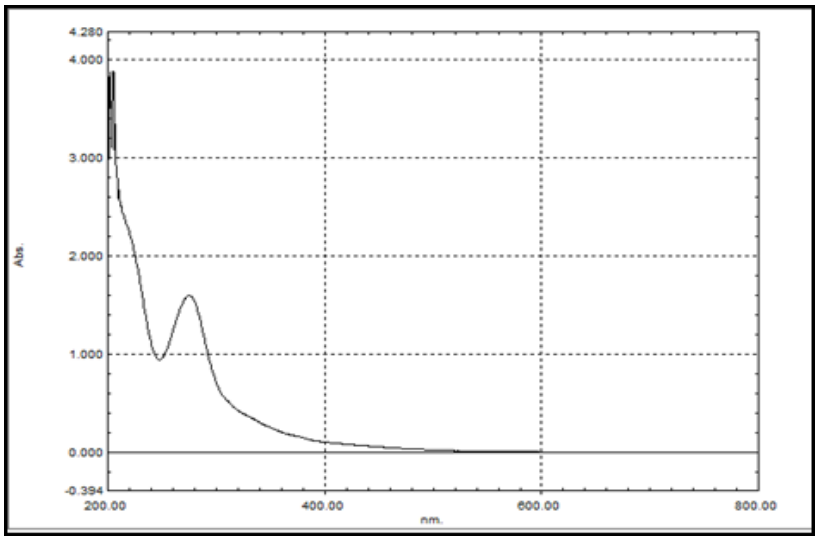

Fig 4: UV-Spectrum Capsaicin

Table 1: Developed method parameters

Instrument name	UV- Visible Spectrophotometer
Make	Sican2301,
Solvent	Methanol
Wavelength of Curcumin	421 nm
Wavelength of Capsaicin	280 nm
Concentration of Curcumin	1-5 µg/ml
Concentration of Capsaicin	25-125 µg/ml

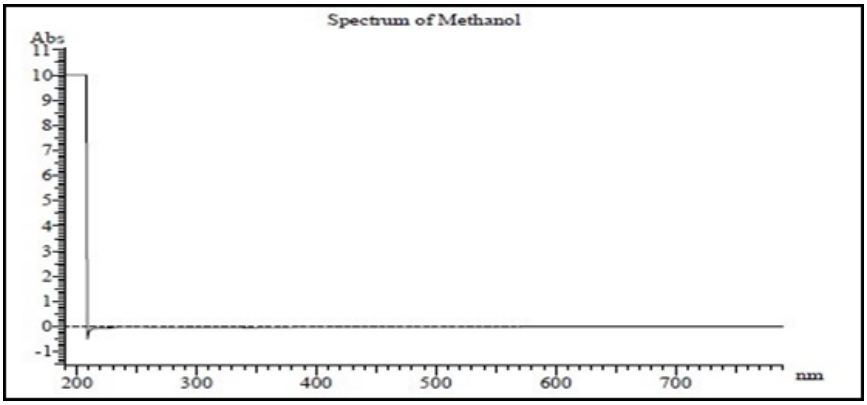

Fig 5: UV-Spectrum methanol

Table 2: Linearity and Range data of Curcumin and Capsaicin

Curcumin		Capsaicin		Statistical Parameters	Curcumin	Capsaicin
Conc.	Abs	Conc.	Abs			
1 µg/ml	0.149	25 µg/ml	0.194	Corr. coefficient	0.998	0.998
2 µg/ml	0.308	50 µg/ml	0.356	Slope	0.161	0.007
3 µg/ml	0.482	75 µg/ml	0.519	% Curve fitting	99.80%	99.80%
4 µg/ml	0.637	100 µg/ml	0.685	LOD	0.225	7.380
5 µg/ml	0.825	125 µg/ml	0.895	LOQ	0.680	22.365

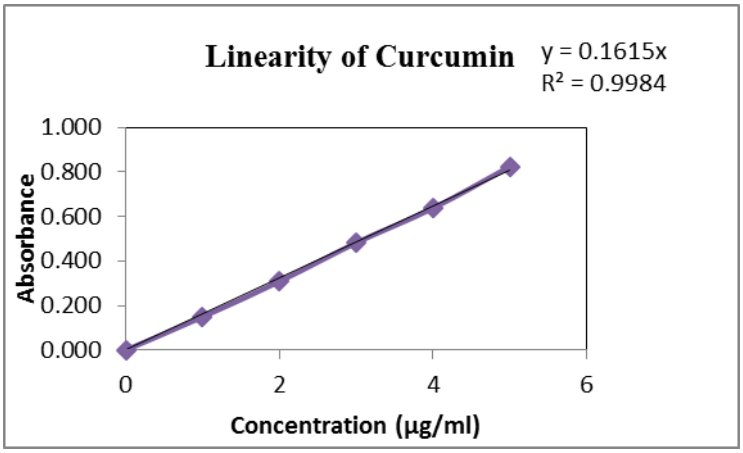

Fig 6: Standard calibration curve of Curcumin

Table 3: System and intra-day precision data of Curcumin

Precision	System	Intra-day 1	Intra-day 2	Intra-day 3	Intra-day 4
Replicates	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
1	0.433	0.436	0.431	0.425	0.439
2	0.446	0.429	0.435	0.434	0.456
3	0.442	0.424	0.442	0.448	0.438
4	0.456	0.439	0.438	0.440	0.440
5	0.450	0.440	0.438	0.445	0.449
6	0.454	0.443	0.445	0.436	0.447
%RSD	1.901%	1.666%	1.131%	1.888%	1.592%

Table 4: System and intra-day precision data of Capsaicin

Precision	**System**	**Intra-day 1**	**Intra-day 2**	**Intra-day 3**	**Intra-day 4**
Replicates	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
1	0.546	0.502	0.498	0.495	0.506
2	0.549	0.498	0.515	0.497	0.493
3	0.53	0.496	0.518	0.511	0.496
4	0.533	0.510	0.523	0.502	0.519
5	0.538	0.512	0.522	0.504	0.51
6	0.544	0.517	0.514	0.519	0.511
%RSD	1.401%	1.661%	1.763%	1.784%	1.935%

Table 5: Interday precision data of Curcumin and Capsaicin

Analytes	**Curcumin**			**Capsaicin**		
Precision	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
Replicates	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
1	0.430	0.425	0.438	0.497	0.502	0.422
2	0.441	0.431	0.429	0.498	0.495	0.434
3	0.443	0.438	0.447	0.519	0.510	0.430
4	0.451	0.441	0.441	0.501	0.518	0.427
5	0.449	0.445	0.437	0.509	0.517	0.425
6	0.447	0.442	0.446	0.516	0.520	0.413
%RSD	1.710%	1.731%	1.506%	1.863%	1.962%	1.705%

Table 6: Robustness data of Curcumin and Capsaicin

Analytes	**Curcumin**		**Capsaicin**	
Replicates	Abs. 420 nm	Abs. 422 nm	Abs. 279 nm	Abs. 281 nm
1	0.446	0.440	0.448	0.436
2	0.437	0.432	0.447	0.441
3	0.439	0.443	0.444	0.446
4	0.450	0.442	0.442	0.427
5	0.441	0.436	0.441	0.426
6	0.435	0.433	0.445	0.440
%RSD	1.287%	1.068%	0.616%	1.841%

Table 7: Ruggedness data of Curcumin and Capsaicin

Ruggedness	**Change in Analyst**		**Change in Instrument**		**Change in Solvent Make**	
Replicates	Curcumin	Capsaicin	Curcumin	Capsaicin	Curcumin	Capsaicin
1	0.425	0.495	0.439	0.497	0.313	0.498
2	0.429	0.499	0.442	0.517	0.315	0.484
3	0.440	0.518	0.429	0.496	0.307	0.483
4	0.437	0.506	0.445	0.509	0.312	0.495
5	0.443	0.510	0.448	0.516	0.305	0.483
6	0.447	0.512	0.451	0.518	0.321	0.504
%RSD	1.923%	1.682%	1.761%	1.979%	1.839%	1.846%

Table 8: Solution Stability

Stability in hours	**Abs of Curcumin at 421 nm**	**Abs of Capsaicin at 280 nm**
1	0.408	0.478
24	0.410	0.476
48	0.410	0.477
96	0.412	0.479
120	0.413	0.480
144	0.410	0.481
168	0.412	0.481
192	0.414	0.483
216	0.415	0.485
264	0.412	0.484
288	0.410	0.486
312	0.410	0.482
336	0.409	0.482
360	0.410	0.480

Table 9: Method Validation report of Curcumin and Capsaicin

Method Validation report of Curcumin and Capsaicin			
Analytes		Curcumin	Capsaicin
Linearity and range		1-5 µg/ml	25- 125 µg/ml
Specificity and Selectivity		No interference of solvent at maximum absorbance of both analytes	
System precision		1.901%	1.401%
Intraday precision		1.569%	1.785%
Interday precision		1.649%	1.843%
Robustness	Lower λ Max	1.287%	0.616%
	Higher λ Max	1.068%	1.841%
Ruggedness	Change in Analyst	1.923%	1.682%
	Change in Instrument	1.761%	1.979%
	Change in Solvent Make	1.839%	1.846%
Limit of detection		0.225 µg/ml	7.380 µg/ml
Limit of quantification		0.680 µg/ml	22.365 µg/ml

Conclusion

The UV Spectrophotometric technique was developed for the simultaneous estimation of Curcumin and Capsaicin in bulk and validated according to ICH guidelines. The developed method was found to be Novel, simple, selective, specific, precise, reliable and reproducible. All results were found to be within the acceptance limit as per ICH guidelines. Hence it can be easily used for the routine quality control analysis of combined Curcumin and Capsaicin in bulk

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